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Specification and Drawings as originally filed, with Application for Patent Serial No:
2,382,768, on April 19, 2002, by **UNIVERSITÉ DE SHERBROOKE**, assignee of
Adrien Beaudoin and Ouhida Benrezzak, for "A Method for the Treatment of Lymphocytic
Disorders and Therapeutic Approach".

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Part 1 – Introduction

ATP and ADP act as signaling molecules for cells of virtually all origins. Indeed these purines, which are released from the cells by exocytotic and non-exocytotic mechanisms can interact with specific receptors and thereby can influence the different physiological systems. One finds on the cell surface two classes of receptors identified as purinoceptors which respond to ATP and ADP and one additional class of purinoreceptors which respond to adenosine. In the immune system the role of purines is still poorly understood. It is well known however that extracellular ATP can modulate the responses of various lymphocyte cell populations such as DNA synthesis, blastogenesis and mediated cell killing via specific cell membrane purinergic receptors. In these respects, it has been shown that ATP inhibits natural killer cell activity (11) of human and murine origins and phagocytosis in mouse macrophages (3). Extracellular ATP can stimulate in vivo DNA synthesis of thymocytes but inhibits DNA synthesis of spleen and peripheral blood lymphocytes (6). Baricordi et al (7), reported that ATP had a synergistic effect on DNA synthesis stimulated by selective T-cell mitogens such as PHA or anti-CD₃ monoclonal antibody.

At least two functionally distinct receptors subtypes have been described in lymphocytes: ① a G protein coupled P₂Y receptor linked to IP₃ generation and Ca²⁺ mobilization from intracellular stores, and ② P_{2x}/P_{2z} receptor which gates a channel permeable to Na⁺ and Ca²⁺ (7). A P_{2y} receptor has been described in human B lymphocytes and has been reported to be absent from T cells (13-17). Whereas nucleotide activated ion channel has been shown to be expressed to a low level in normal B-lymphocytes and to be upregulated in resting mouse T and B-lymphocytes, leukemic peripheral blood lymphocytes. According to Baricordi et al. (7) human peripheral blood lymphocytes and purified T lymphocytes express a P₂ x₇ purinergic receptor and ionic channel gated by extracellular ATP that is involved in the control of mitogenic stimulation by different stimuli. The extracellular concentration of the agonist (ATP, ADP or adenosine) which elicits the cellular response is determined by several parameters: i) rate of release and diffusion, ii) metabolism by ectonucleotidases and iii)

binding affinity of the receptors. In this context the role played by ectonucleotidases and more specifically NTPDase appears to be determinant. In this respect immuno competent cells, such as lymphocytes and macrophages express ectonucleotidases activities. Ectonucleotidase which catalyses the hydrolysis of ATP to ADP has been reported to B cells (18), macrophages (20), NK cell and CTL (21). It has been proposed that an ecto ATPase could protect murine CTL cells from the lytic effects of extracellular ATP released during granules exocytosis (1,10,22) and that ecto this ATPase was required for the cytolytic activity of NK cells. Recent data ATPDase show that NTPDase 1 (CD₃₉), which is distributed on the cell surface of many cell types, plays a key role in the conversion of extracellular ATP to ADP and ADP to AMP. This enzyme, put in evidence many years ago by Lebel et al. (36) in pig pancreas, have been recently identified in primary and secondary lymphoid organs including spleen, thymus, tonsils, and Peyer's patches and isolated lymphocytes and macrophages from pig spleen (Benrezzak et al. (1999)). It has also been demonstrated that CD₃₉, a lymphoid cell activation antigen (30), corresponds to human NTPDate (31). Kansas et al. (32) cloned the latter molecule and studied its distribution and they reported that this protein is expressed on activated NK, B, and T cells of peripheral blood and is found in lymphoid tissues namely tonsils and thymus. Despite these reports demonstrating the NTPDase (CD₃₉) in the immune system, the physiological role played by the NTPDase is the immune response remains to be clarified. A relevant obstacle to the understanding of the NTPDase functions is a lack of specific inhibitors, i.e. an inhibitor that does not interfere with purinoceptors. A recently described NTPDase inhibitor BGO 136, also known as 1-hydroxynaphtalene-3,6 disulfonic acid, produced a mixed type of inhibition with K_i s of 380 and 380uM with ATP and ADP as substrate respectively. Pharmacological characterization of BGO 136 for possible purinergic activities showed antagonistic effect on P_{2y2} receptor responses to UTP (Gendron, Benrezzak et al. (2002), in press Drug targets; Biochemical and pharmacological characterisations of a new NTPDase inhibitor : the (8 Bus ATP) are described in Gendron et al. (2000), J Med Chem). In this study, the influence of the NTPDase inhibitor on cellular and/or humoral immune responses was examined. More specifically, the effects of BGO 136 erythrosin, 8 Bus-ATP, 8 Bus-AMP on cell specific mitogenic stimulation and on primary

antibody response to a T-cell dependent antigen are reported. These findings emphasize the importance of NTPDase for the T-helper cell for functions in humoral responses induced by T cell dependent antigens.

NB : References appear in paper Benrezzak et al. (1999)

The NTPDase inhibitors and T cell lymphoproliferative disorders : a possible approach to therapy of blood disorders.

Based on our results which showed that the NTPDase inhibitors suppress the lymphoproliferative properties of T cells, we propose a strategy to therapy of blood disorders, particularly for neoplasia (lymphoma-leukemia syndrome).

The T cell leukemia is a T cell disorder : a progressive or aggressive disease. The T cell malignancies can be classified into two principal groups : 1-T cell acute lymphoblastic leukemia (ATLL) and 2-T cell chronic lymphoblastic leukemia.

Actual clinical therapies include chemotherapy, radiotherapy, bone marrow transplantation, or a combination of this treatment. Patients are almost always resistant to chemotherapeutic agent and were refractory to other therapies. ATLL (adult T cell leukemia lymphoma) is a very aggressive T cell malignancy for which no successful treatment is yet available. The acute form have a poor outcome with overall survival ranging from 5 to 13 months. ATLL does not respond or only transiently to combination chemotherapy.

Patient with chronic forms becomes refractory to therapy. The poor response to therapy relates to chemotherapy resistance. Results are not superior with immunotherapy.

Based on our results obtained on mouse which revealed efficacy of NTPDase inhibitors and tolerance for long-term treatment, we propose the use of NTPDase inhibitors as a

new approach or strategy to the treatment of blood disorders such as neoplasia (leukemia and lymphoma).

Part 2 – Description of results

Example 1

Presence of NTPDase as demonstrated by Western-blot on different types of lymphoid cells (Normal or neo-plastic).

Notice that the signal is more pronounced in neo-plastic cells as compared to normal peripheral blood lymphocytes (PBL). (Figure 1)

In parallel with these blots the levels of ecto-ATPase and ecto-ADPase activities are presented in Table 1. In agreement with the Western-blot the levels of ectonucleotidase activity in normal PBL cells is relatively low as compared to all other tumor cell lines. It is noteworthy that in most cases 10 mM Na azide (an inhibitor) cause more than 50% inhibition of enzyme activity.

Example 2

In a recent study it was shown that BGO 136 is an efficient spleen NTPDase inhibitor (Gendron et al. 2001. Submitted). As shown in figure 2 with PBL, BGO causes a dose-dependent inhibition of both ATPase and ADPase activities. In both cases a significant inhibition (40%) is obtained with 5 mM BGO 136 whereas with 10 mM it increases up to more 50% or more. The results are expressed as percent of controls considering untreated control samples as 100.

Influence of BGO on human lymphocyte proliferation stimulated by ConA and LPS is shown in figure 3. BGO does not affect the proliferation of non-activated lymphocytes as measured by ³H-Thymidine incorporation in DNA. In contrast proliferation induced

by ConA is inhibited in a dose-dependent manner, reaching about 18% of the positive control values with 10 mM BGO 136. The BGO 136 inhibition of LPS induced proliferation was much less pronounced but one has to take into account that cell-induced proliferation as expected is relatively low.

These results led us to consider how BGO 136 would influence the humoral response in-vivo since the latter is dependent upon T cell activation.

Example 3

The influence of the different NTPDase inhibitors was tested on the production of antibodies (humoral immune response) in the mouse. As shown in figure 5 the levels of albumin antibodies was reduced as much as 50% by a single dose BGO 136 (800mM). Repeated injections of BGO 136 appeared even more efficient. (see group named DM)

The experiments were repeated in several series of mice and produced essentially the same pattern of inhibition. The results from one representative experiment are presented in figure 5.

A positive correlation (.89 for ATPase and .96 for ADPase) between cell proliferation and NTPDase activity was established in the presence of the three concentrations of BGO 136 is illustrated in figure 4.

Example 4

The experiments of example 2 were repeated with a different type of inhibition erythrosin non-structurally related to BGO 136.

Results shown in figures 6-7-8 show a dose-dependent inhibition of lymphocyte proliferation (Figure 7) correlated with NTPDase activity (Figure 6). See figure 8 for the correlation.

Example 5

The experiments of example 3 were repeated with erythrosin as shown in figure 9. A significant decrease in the levels of antibodies to albumin can be observed especially with multiple injections to sustain the concentration of the inhibitor in the animal. (see group named DM, details in legend of methods)

Example 6

The experiments of example 2 were repeated with 8 BUS-ATP (Figures 10-12) and 8 BUS-AMP (Figures 13-15).

In both cases a pronounced dose-dependent inhibition of the NTPDase activity was observed. These inhibitors reduced cell proliferation induced by ConA in a similar manner. Moreover a very good correlation could be established between these two parameters (Figures 12, 15).

Example 7

Similar experiments were repeated also with β - γ -Me ATP and as shown in figure 16 NTPDase was inhibited in a dose-dependent manner. In parallel the inhibition of cell proliferation was observed (Figure 17) and again the correlation was established (Figure 18).

Example 8

Having established the influence of the enzyme on the proliferation of normal lymphocytes we examined the influence of the different inhibitors on Jurkat cells which are derivatives from human leukemia cells as shown in the different panels of figure 19. BGO 136, erythrosin, 8 BUS ATP and even AMP (the product of ATP hydrolysis by

NTPDase) exerted a marked reduction of cell proliferation. A less pronounced but significant effect was observed with β - γ -Me ATP.

Part 3 – Description of methods

Claims

- 1- A method to inhibit the activation and proliferation of normal neoplastic cells of the immune system of mammalian.
- 2- The method of claim 1 where the inhibitor blocks the NTPDase activity.
- 3- The method of claim 2 where the NTPDase inhibitor is BGO136 or a member of this family.
- 4- The method of claim 2 where AMP is the NTPDase inhibitor.
- 5- The method of claim 2 where the NTPDase inhibitor is an ATP analogue (8 Bus-ATP, 8 Bus-AMP).
- 6- The method of claim 2 where the NTPDase inhibitor is erythrosine B or a member of this family.
- 7- The method of claim 1 where the immune cell is a T lymphocyte.
- 8- The method of claim 1 where the immune cell is a B lymphocyte.
- 9- The method of claim 1 where the cell is a neoplastic cell of the immune system.
- 10-The method of claim where the production of antibodies is reduced in vivo.
- 11-The method of claim 10 where the activation and proliferation occur following organ transplantation.
- 12-The method of claim 10 where the animal is immunologically stimulated by an allergen.
- 13-The method of claim 10 where the immune system is naturally stimulated as in autoimmune diseases.

Experimental methods used to obtain results described here

◦ **Description**

◦ **Human lymphocytes**

Human lymphocytes were obtained from peripheral venous blood of normal and healthy medication-free volunteers. Fresh blood collected in EDTA glass tubes was layered into Histopaque – 1077 (a solution of Ficoll and sodium diatrizoate adjusted to a density of 1.077, Sigma, USA) and centrifuged at 400 g for exactly 30 min, at room temperature. During centrifugation, erythrocytes and granulocytes are aggregated by Ficoll and rapidly sediment, whereas lymphocytes remain at the plasma interface. Plasma (the upper layer) was carefully removed to prevent disturbance of the buffy coat. The latter fraction rich in lymphocytes was recovered with a pasteur pipette and washed twice with RPMI 1640 medium by centrifugation, for 10 min, at 250 x g. The final pellet was suspended in fresh RPMI 1640 medium supplemented with 2mM L-glutamine, 10% foetal bovine serum inactivated at 56°C for 30 min, and antibiotics: penicillin 100 units/ml, streptomycin 100 ug/ml and amphotericin 2.5 ug/ml. Cells were counted with the hemacytometer. Viability tested with Trypan blue exclusion assay, was superior to 90%. The cell preparation named PBL (for peripheral blood lymphocyte) was immediately used for NTP Dase assays and for mitogenic responses to stimulators.

◦ **Human cell lines**

Human cell lines used in the present investigation were given by Dr Jana Stankova (Department of Immunology, Université de Sherbrooke). Jurkat cells are derived from human T cell leukemia, Raji and Ramos are B-cell lymphomas derived from peripheral blood of patients with Burkitt lymphoma. MonoMac-1 is a cell line derived from the peripheral blood of a 64 year-old male with active monocytic leukemia. K562, which is used as highly sensitive target for NK cell activity is an erythroleukemia, cell line. U937

is a promonocytic myeloid cell and finally the Y₂ T₂ C₂ (NY) cell is a human natural killer cell line. These lymphoblastoid cell lines which grow in suspension as single cells without attachment to glass, were maintained by passage in complete RPMI 1640 medium with 2 mM L-glutamine containing 10-20% heat inactivated foetal bovine serum [V/V] and antibiotics (100 units/ml of penicillin), streptomycin 100 ug/ml and 2.5 ug/ml of amphotericin) at 37°C in a humidified 5% CO₂/95% air atmosphere. Mono-Mac 1 cells were maintained in the same complete RPMI 1640 medium supplemented with 1 x non-essential amino acids and 1mM Na-pyruvate at 37°C with 5% CO₂.

• Mitogen stimulation and proliferation assay

A thymidine uptake assay was used to evaluate the effect of inhibitors on the proliferative activity of normal lymphocytes cells.

Cell suspensions of fresh human peripheral blood lymphocytes (PBL) were resuspended in RPMI 1640 medium supplemented with 10% foetal bovine serum at a concentration of 2×10^6 /ml. Aliquots of 100 ml were plated on a Falcon flask (96 – well) and stimulated with 10 ug/ml of (Con-A) or 40 ug/ml of (LPS) at a final volume of 0.2 ml/well. ConA and LPS are used to stimulate preferentially T cells and B cells respectively. Cell cultures \pm inhibitors were incubated for 48 h at 37°C in a 5% CO₂ atmosphere [³H] thymidine (1 ug/well, specific activity 5.0 ci/mmol) was added to each microplate in a volume of 50 ul. After an additional a 4 h incubation at 37°C, cells were collected with a cell harvester and ³H thymidine incorporation was measured in triplicate samples by liquid scintillation counting.

• NTPDase assays

Prior to the assays, human normal lymphocytes as well as leukemic cell lines, were washed three times with phosphate-free saline in 95 mM NaCl, 1mM CaCl₂, 2mM MgCl₂ and 45mM Tris – HCl buffer (pH 7.4). Enzyme assays were carried out at 37°C in 1 ml

of the following assay medium: 95mM NaCl, 5mM KCl, 1mM CaCl₂, 2mM MgCl₂, 5mM glucose, 0.05% BSA, 5mM tetramisole and 45mM Tris-HCl (pH 7.5). The reaction was started by adding the substrate (200uM of ATP or ADP) and stopped with 250 ul of the Malachite green reagent. Controls were run with the enzyme added after the Malachite green reagent. Where indicated, 10mM sodium azide (NaN₃) or 1mM, 5mM and 10 mM of BGO 136 were added directly to the assay mixture. Inorganic phosphate was estimated according to Baykov et al.⁽¹⁾ and enzyme activity was either expressed as nmoles of Pi released/min/mg/ of protein which corresponds to mUnits ⁽²⁾ or as nmoles Pi/min/10⁶ cells. Where were indicated, lymphoid cells were lysed by three freeze thaw cycles in saline buffer and protein was measured with the method of Bradford using bovine serum albumin as a standard ⁽³⁾.

• Electrophoresis and Western blotting

Lymphocytes from human peripheral blood (PBL) and lymphoblastoid cell lines obtained as described above were lysed and protein was measured. Samples of 20 ug protein of whole cell lysate were applied to each well of polyacrylamide gel. Electrophoresis was carried out under denaturing conditions (SDS-PAGE) in a 10% polyacrylamide gel. Protein was subsequently transferred to immobilon-P sheets and immunoblotted. A rabbit antiserum (Kally) which recognizes isoform II of ATPDase was used as primary antibody at a dilution of 1:10 000. The secondary antibody, a mouse monoclonal anti rabbit IgG conjugated to alkaline phosphatase (1:10 000) was detected by chemiluminescence using the Immun-Star substrate, according to the recommendations of the supplier (Bio-Rad/laboratories).

(1), (2), (3) - See Benrezzak et al. 1999. Archives of biochemistry and biophysics, 370(2) : 314-322.

◦ **In vivo antibody production assay**

Animal treatments and experimental design

Animal treatments and experimental design. Immunity mice were primed with antigen by an intraperitoneal injection of BSA as a T-dependent antigen. Briefly BSA was resuspended in PBS (pH 7.2) and emulsified in the same volume of complete Freund's adjuvant (CFA). 30 ug BSA in 100 ml PBS was given to each mouse. Four groups of ten mice were immunized.

- Group 1 :** mice were injected i.p. with BSA alone with emulsified in (CFA) group: Ag.
- Group 2 :** mice were injected i.p. with BSA plus BGO 136 (400mM) in 100 ml PBS, emulsified in (CFA) or erythrosin B (0.25 g/Kg)
- Group 3 :** mice were injected i.p. with BSA plus BGO 136 (800mM) in 100 ml PBS, emulsified in (CFA) or erythrosin B (0.5 g/Kg)
- Group 4 :** mice were treated with BSA in presence of the inhibitor emulsified in (CFA). These mice received various i.p. doses of BGO 136 400mM in 0.1 ml of normal PBS or erythrosin B 0.25 g/Kg, 2 doses/week are given as a maintenance dose. (group named DM = maintenance dose)

These initial immunizations (i.p.) was followed by two subsequent immunizations with emulsions of BSA alone or in presence of BGO136 400mM or 800mM, in incomplete Freund's adjuvant. Five days after each immunization, blood was collected from each animal, sera was analysed by an ELISA assay.

Detection of antibodies

Flat-bottomed microtiter plate were coated with BSA (5ug/ml) in $\text{NaHCO}_3/\text{Na}_2\text{CO}_3$ 50mM (pH 9.6) at 37°C for 2 h. Plates were washed three times with (PBS buffer, pH 7.4, 0.5 ml/L Tween-20) and then blocked with 400 ul of milk per well overnight, at 4°C.

Diluted sera specimens were added (100 ul/well) and incubated for 2h at 37°C. After thorough washing, wells were incubated with 100 ul of goat-anti-mouse IgG conjugated with peroxidase for 2 h at 37°C followed by several washes. TMB substrate solution (100 ul/well), (42mM TMB in DMSO, 0.1 M citric acid, Na₂HPO₄ 0.2 M and H₂O₂ 30%) was added. Color development was allowed to proceed for approximately 15 to 30 min, at room temperature. Reaction was terminated by adding 30ul of 4N H₂SO₄. Optical density was read at 450 nm.

Cell growth and proliferation of Jurkat cell lines

T cell Jurkat is an acute T cell leukemia obtained from the peripheral blood of human. It was purchased from ATCC and was grown and maintained in RPMI 1640 medium containing 10% FBS, 2mM L-glutamine adjusted to contain 1.5 g/L sodium bicarbonate, 4.5 g/L glucose, 10 mM HEPES and 1.0 mM sodium pyruvate. To evaluate the effect of the different inhibitors of NTPDases on growth of Jurkat cells, BGO 136, erythrosin, 8 Bus ATP, AMP and β - γ -Me ATP are added in the RPMI 1640 medium in presence of cells at day = 0. Cells counts and viability of cells are determined on day = 5.

Statistical analysis

Test animals were mice. Studies were also performed on the cultures of peripheral blood lymphocytes of donors and on experimental tumor cell lines. Test results were statistically processed. A one way variance analysis was followed; the Statpack[®] software analysis program was utilized.

* p<0.05

** p<0.01

*** p<0.001

Part 4 – Conclusions

1. BGO 136, a naphthalene derivative suppresses immunological activity of the organism by inhibiting response of humoral and cellular immunity. Specifically, BGO 136 (1.a.) inhibits the activity of the NTPDase enzyme and (1.b.) parallelly decreased the lymphocyte proliferation. (1.c.) A positive correlation was established between the inhibition of the enzyme activity and the proliferation of T lymphocytes by BGO 136.

Effective doses of BGO 136 are in the range of 5 to 10 mM.

2. Administered by different schemes BGO 136 inhibits humoral response by 50% and more. The effect was demonstrated in vivo using a protocol of immunizations. No toxic or allergic reactions were observed during the BGO 136 administration (two months).
3. The comparative evaluation of BGO 136 and different other NTPDase inhibitors tested showed that erythrosin B, 8 Bus ATP, 8 Bus AMP and β - γ -Me ATP decrease (at lower doses) the NTPDase activity and affect the proliferation of T lymphocytes.
4. All the NTPDase inhibitors here tested when administrated in vitro (cell cultures) influence the growth of leukemic T lymphocyte cells.
5. The NTPDase inhibitors can be recommended for tests in the following areas :
 - In transplantology, as an immunodepressant
 - In oncology and oncohematology
 - In autoimmune disorders as agents normalizing the immunological reactivity of the organism (rheumatoid arthritis, psoriasis).

A METHOD FOR THE TREATMENT OF LYMPHOCYTIC DISORDERS
AND THERAPEUTIC APPROACH

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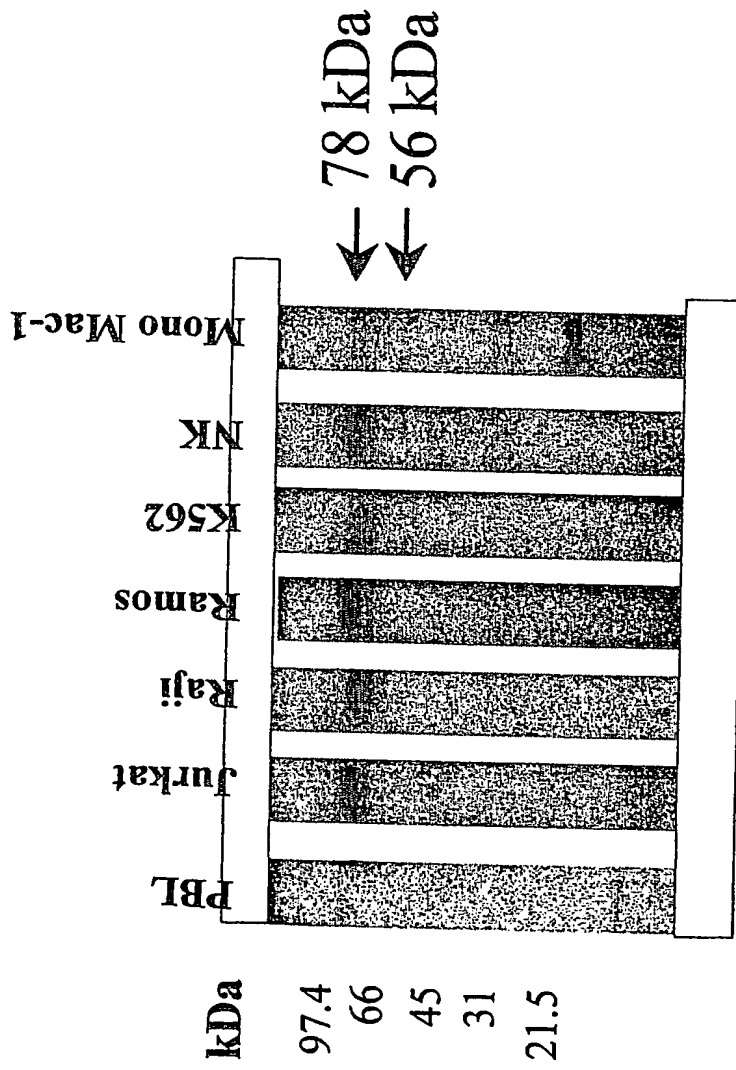
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Presence of NTPDase in human lymphoid cells



PBL : normal peripheral blood cells
 Jurkat : T leukemia cells
 Raji : B leukemia cells
 Monomac : monocytes leukemia cells
 Ramos : B leukemia cells
 K562 : erytroleukemia cells
 NK : natural killer cells

Fig. 1

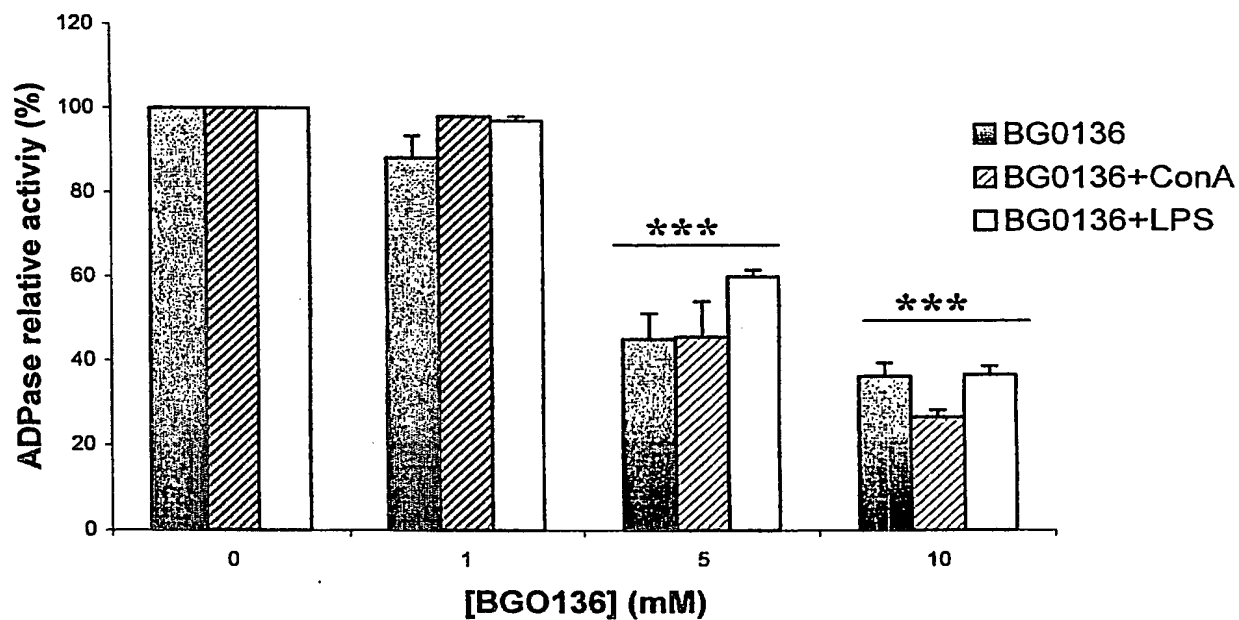
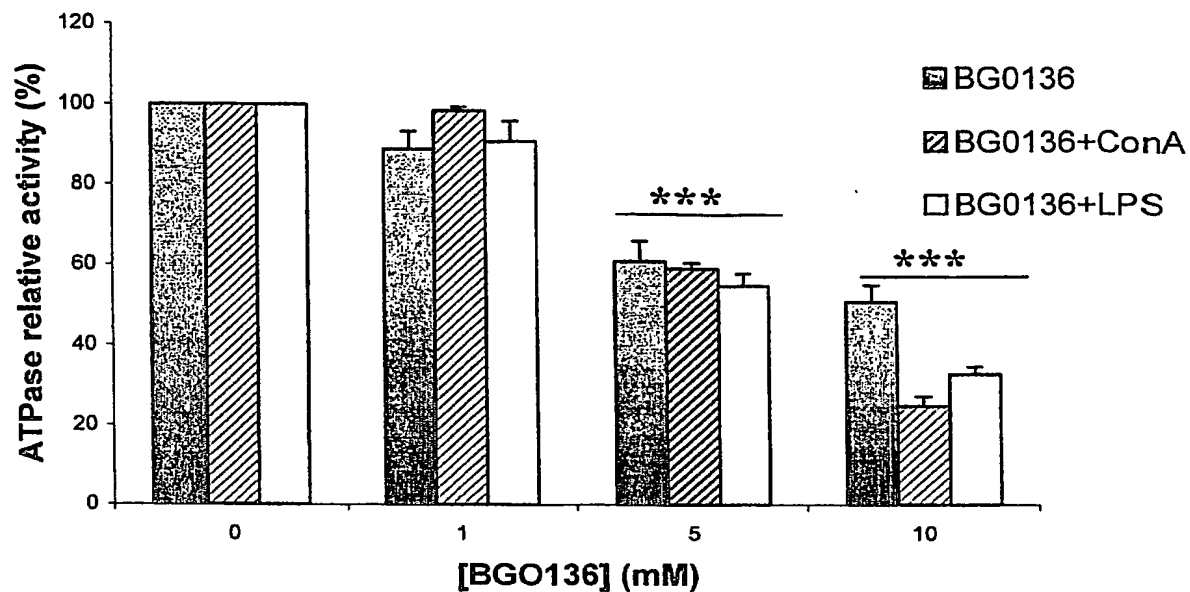
NTPDase activity of the different intact human lymphoid cells

| | (nmoles Pi/min/10 ⁶ cells) | | | |
|------------|---------------------------------------|------------|----------------|------------|
| | ATP | ATP + NaN3 | ADP | ADP + NaN3 |
| Ramos | 20 ± 0.3 *** | 10 ± 0.0 | 14.5 ± 2.4 *** | 8 ± 0 |
| Raji | 39 ± 6.0 *** | 14 ± 0.0 | 21 ± 0.8 *** | 14 ± 0 |
| JurKat | 25 ± 1.5 *** | 7 ± 1.0 | 9.5 ± 0.8 *** | 3.5 ± 0.8 |
| Mono Mac-1 | 23 ± 1.0 *** | 7 ± 1.2 | 7.7 ± 0.2 *** | 3.8 ± 1.8 |
| U937 | 29 ± 1.0 *** | 8 ± 0.0 | 11 ± 0.5 *** | 3.3 ± 0.8 |
| PBL | 1.0 ± 0.1 | 0.5 ± 0.1 | 0.5 ± 0.1 | 0.2 ± 0.1 |

n = number of experiments (each in triplicate)

Table 1

NTPDase activity of activated lymphocytes



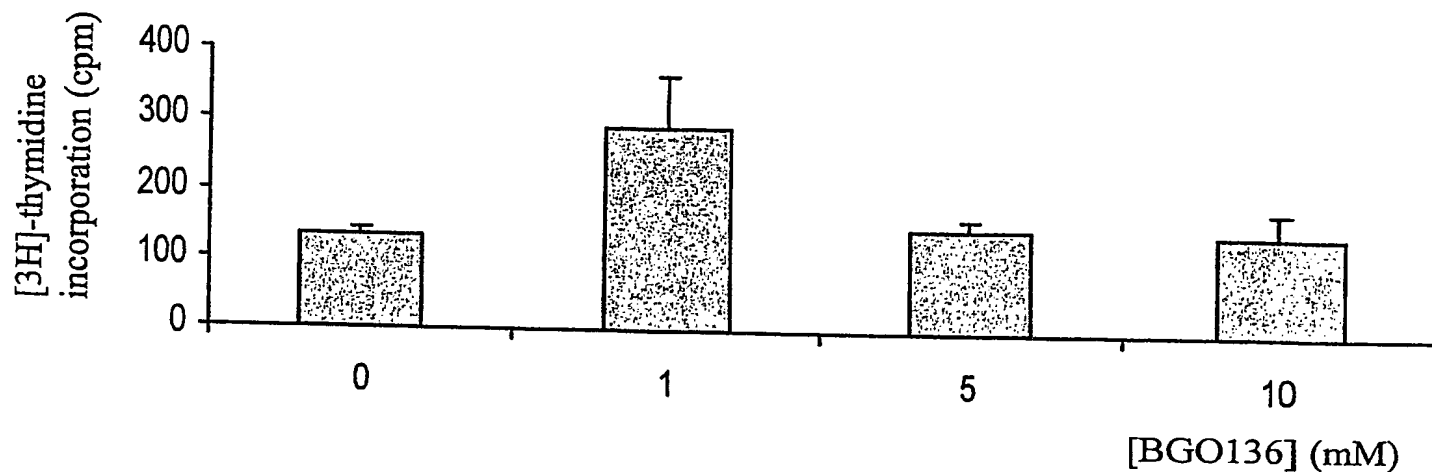
eight (8) assays were performed each in triplicate.

Fig. 2

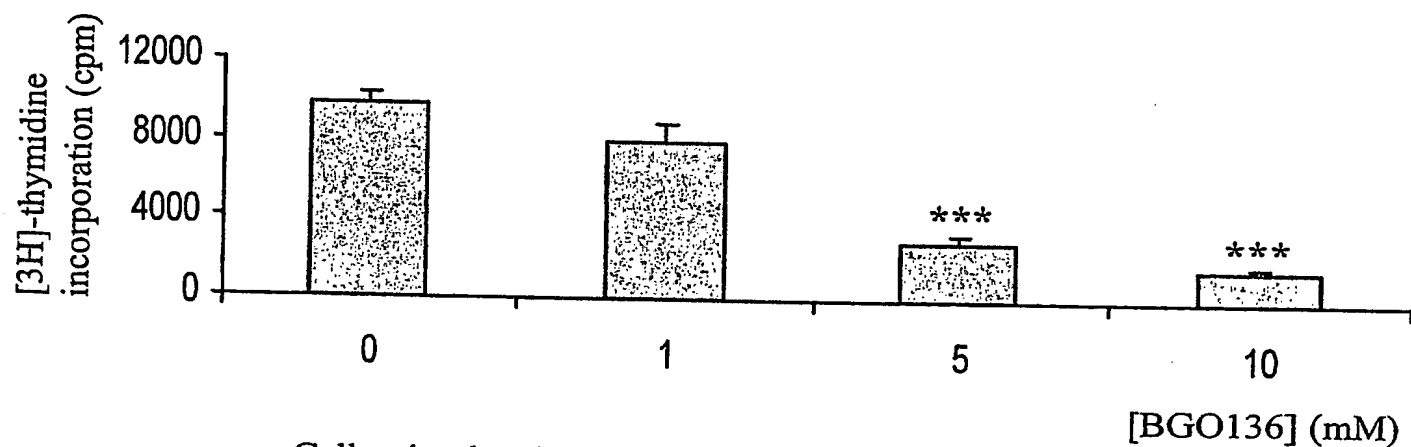
Effect of BGO136 on human peripheral blood lymphocytes (PBL) proliferation

Control

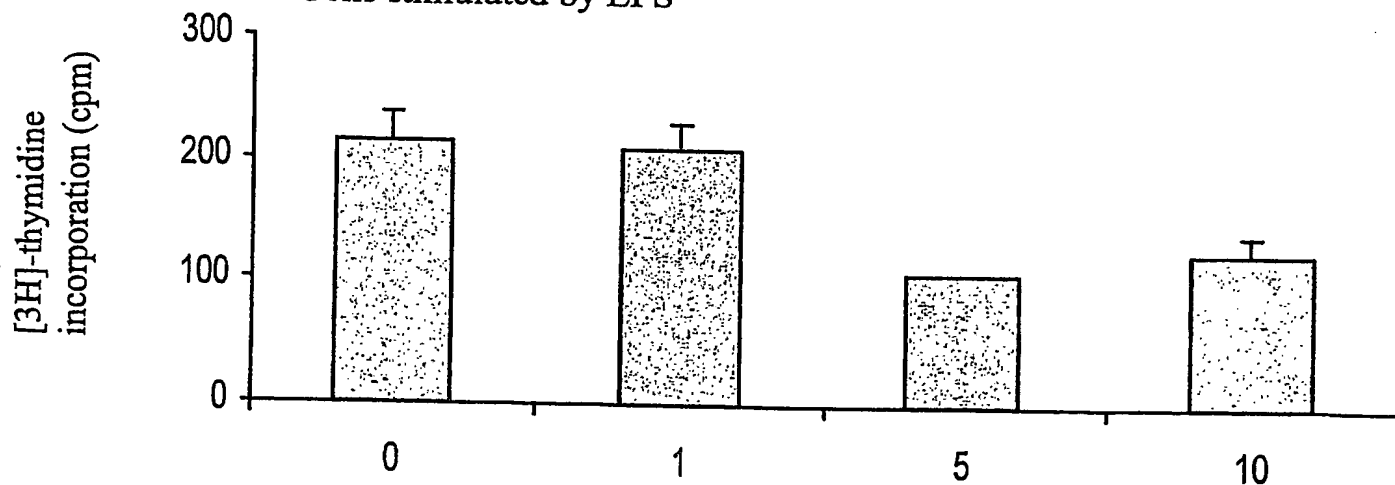
Unstimulated cells.



Cells stimulated by Con A



Cells stimulated by LPS



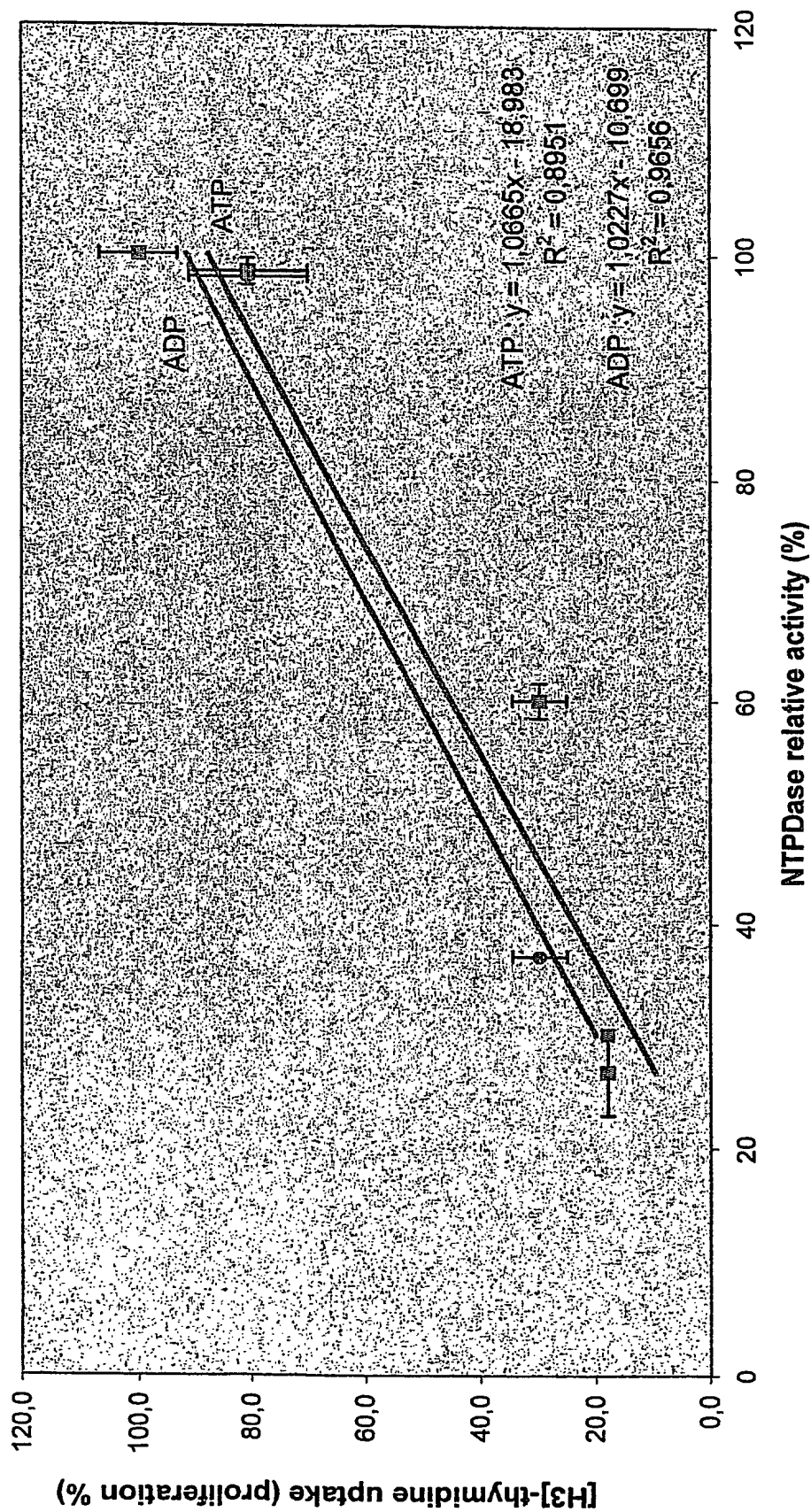
[inhibitor] : indicates inhibitor concentration

[BGO136] (mM)

Three (3) experiments were performed each in quadruplicate

Fig. 3

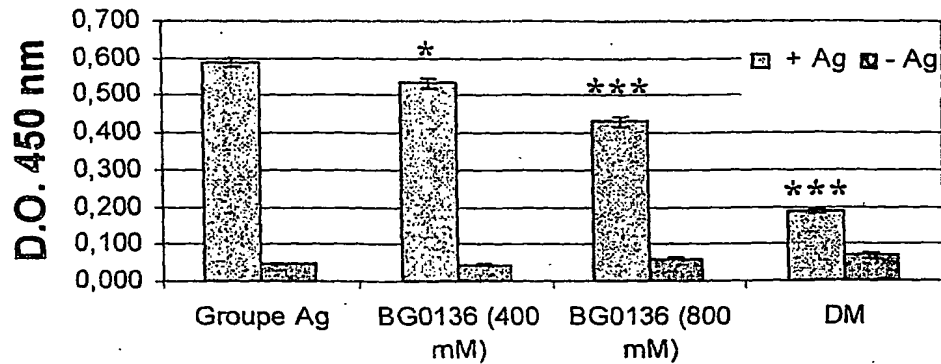
Correlation between NTPDase activity and proliferation assay of peripheral blood lymphocytes (PBL)



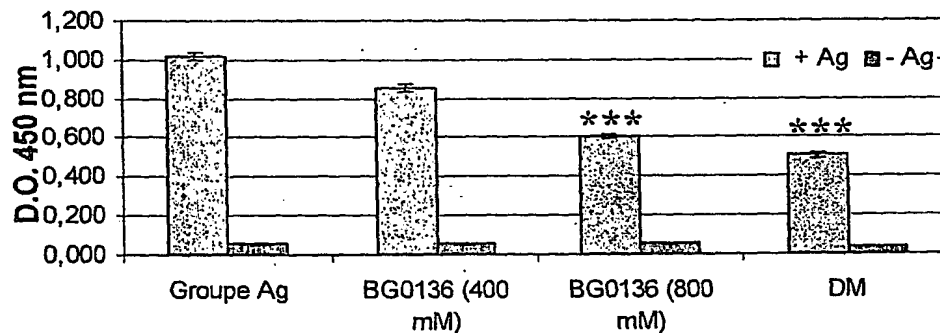
each set of point correspond to a single concentration of the inhibitor (concentrations used are 0, 1, 5 and 10 mM of BGO136)

Fig. 4

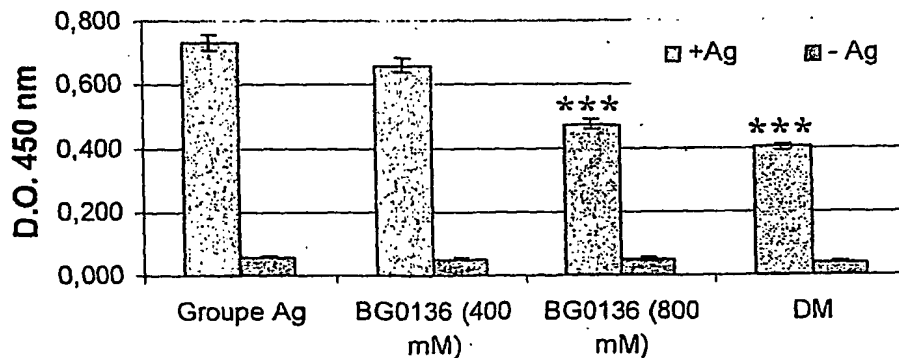
**Effect of BGO136 on humoral response
(antibodies production) 21 days after first injection**



**Effect of BGO136 on humoral response (antibodies
production) 32 days after first injection**



**Effect of BGO136 on humoral response
(antibodies production) 40 days after first
injection**



DM : multiple doses of BGO136 were given (see methods)

+Ag : mice received BSA as Ag T cell dependent.

- Ag : mice received only vehicle

BGO136 was given in a single dose at the mentioned concentration (400 or 800 mM)

Each group contain ten (10) mice

Fig. 5

Effect of Erythrosin B on NTPDase activity of human peripheral blood lymphocytes (PBL)

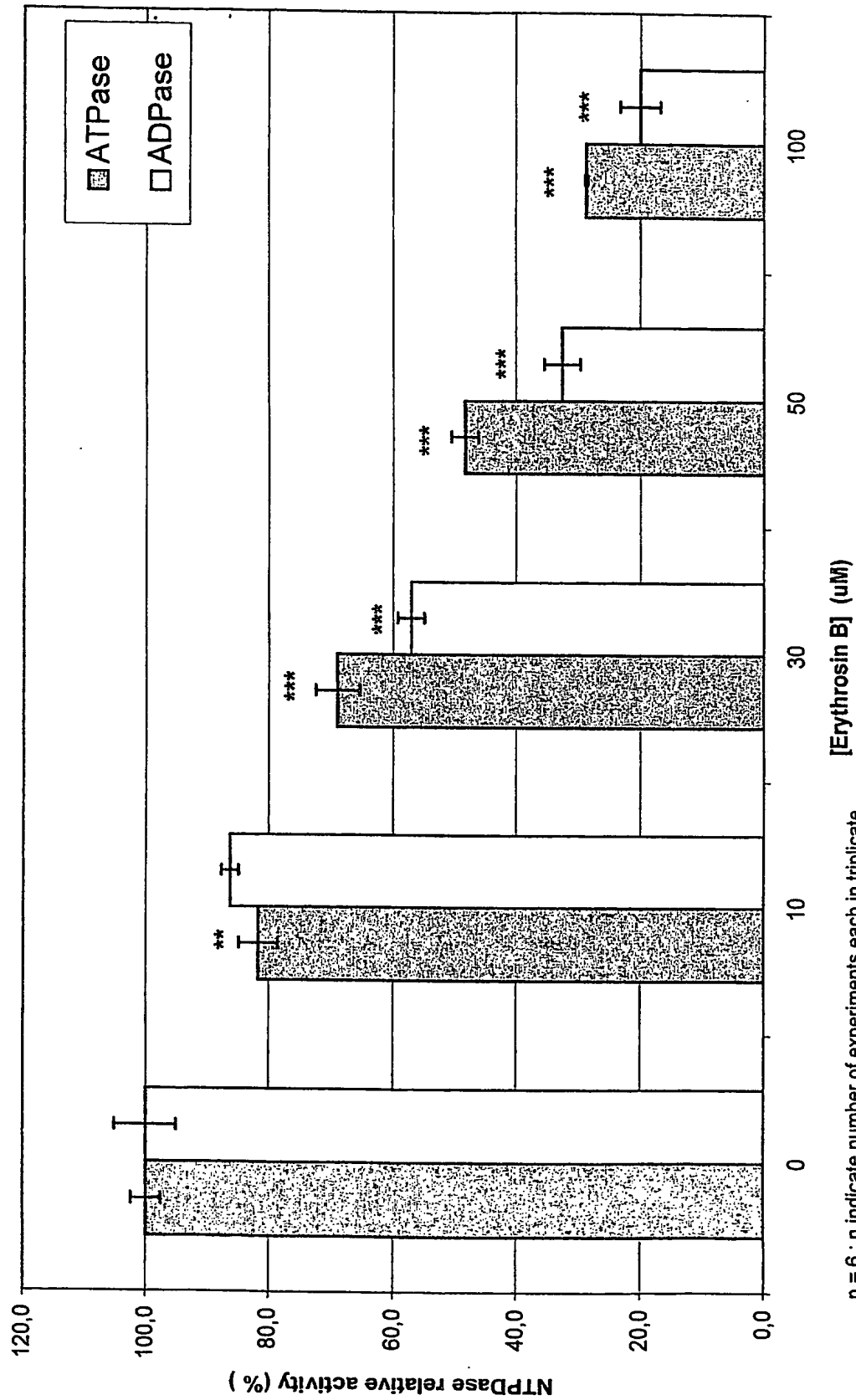
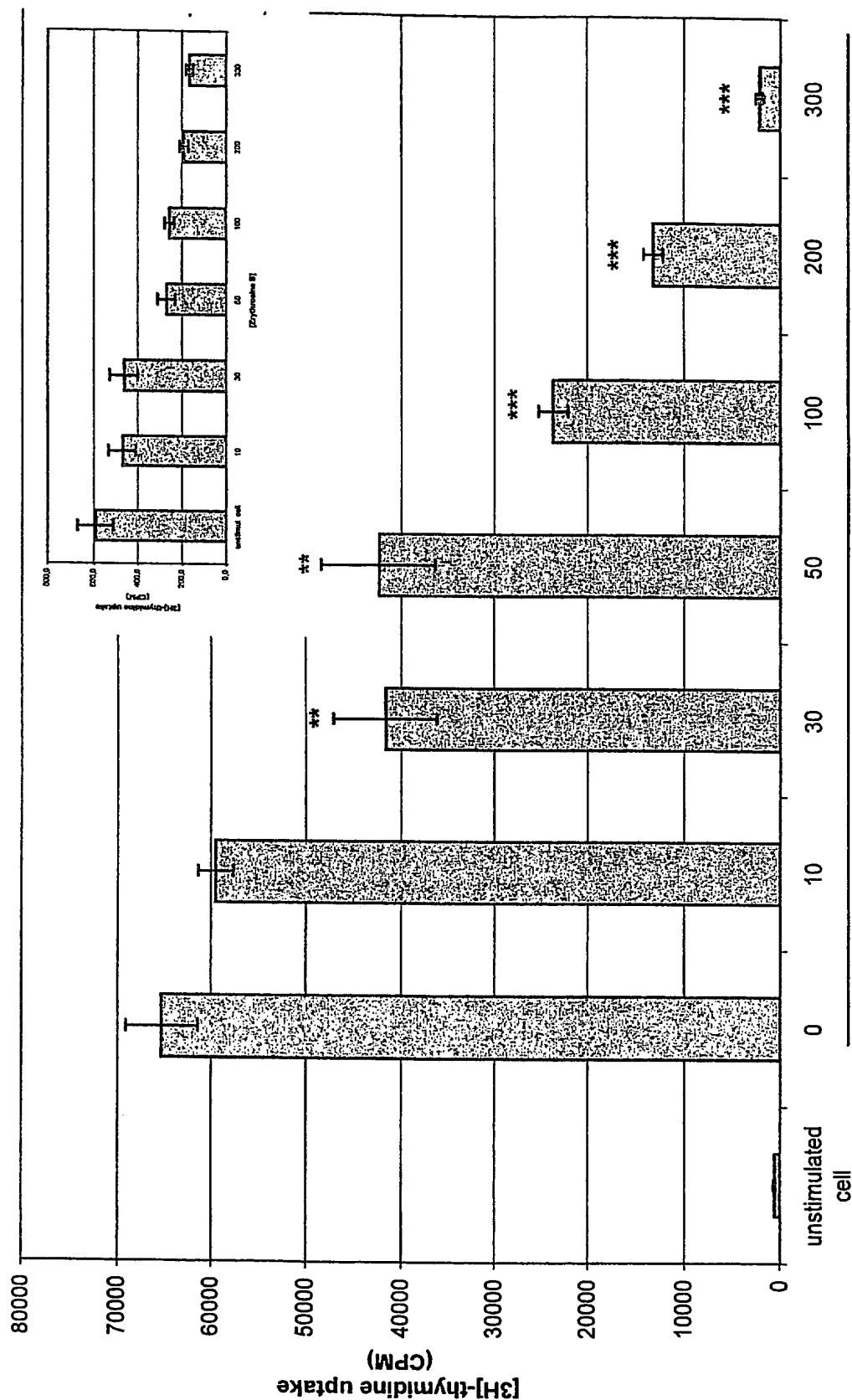


Fig. 6

Effect of Erythrosin B on human peripheral blood lymphocytes (PBL) proliferation

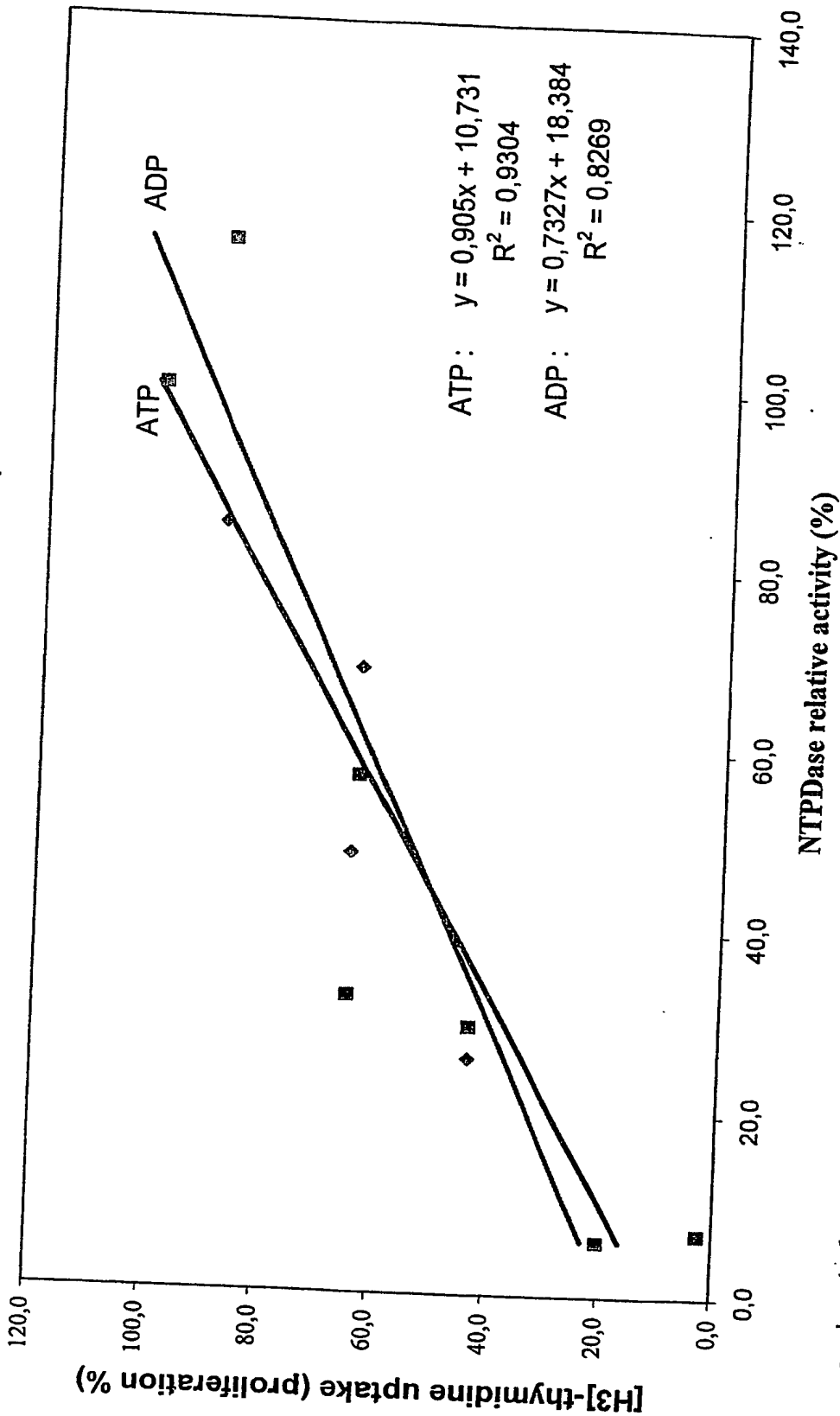


Stimulated cells by Con A +/- [Erythrosin B] (uM)

n = 3 ; n indicate the number of experiments each with sextuplicate

Fig. 7

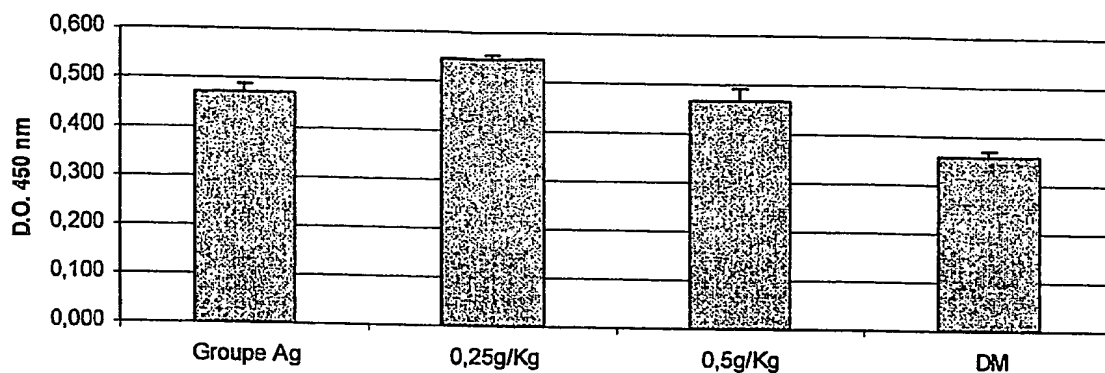
Correlation between NTPDase activity and proliferation assay of peripheral blood lymphocytes (PBL)



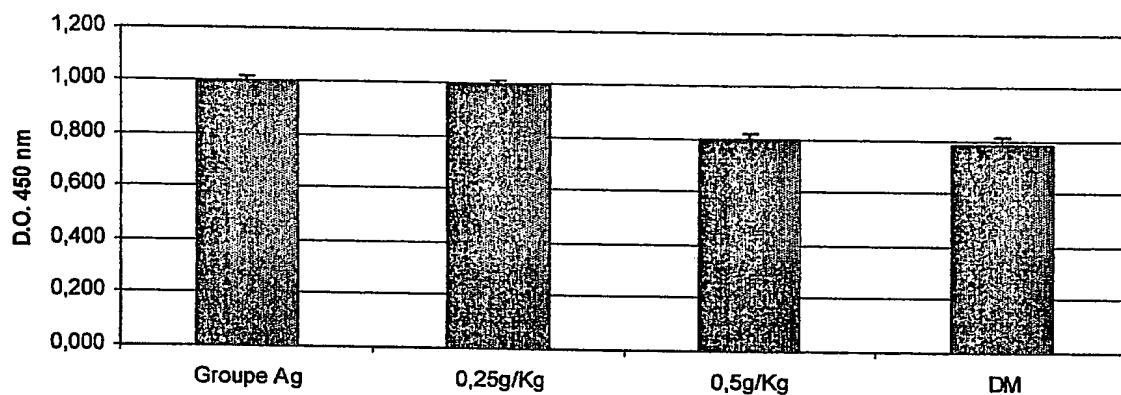
each set of point correspond to a single concentration of the inhibitor (concentrations used are 0, 10, 30, 50 and 100 uM of erythrosin B)

Fig. 8

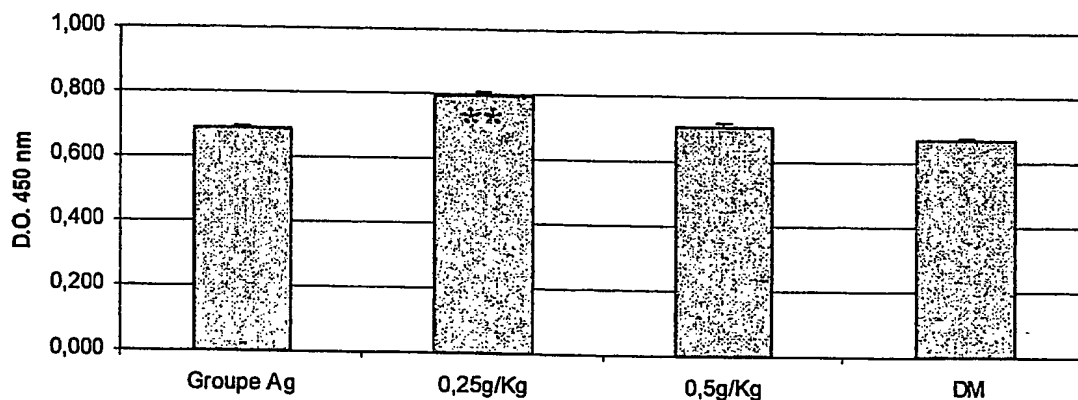
Effect of Erythrosin B on humoral response (antibodies production) 21 days after first injection



Effect of Erythrosin B on humoral response (antibodies production) 32 days after first injection



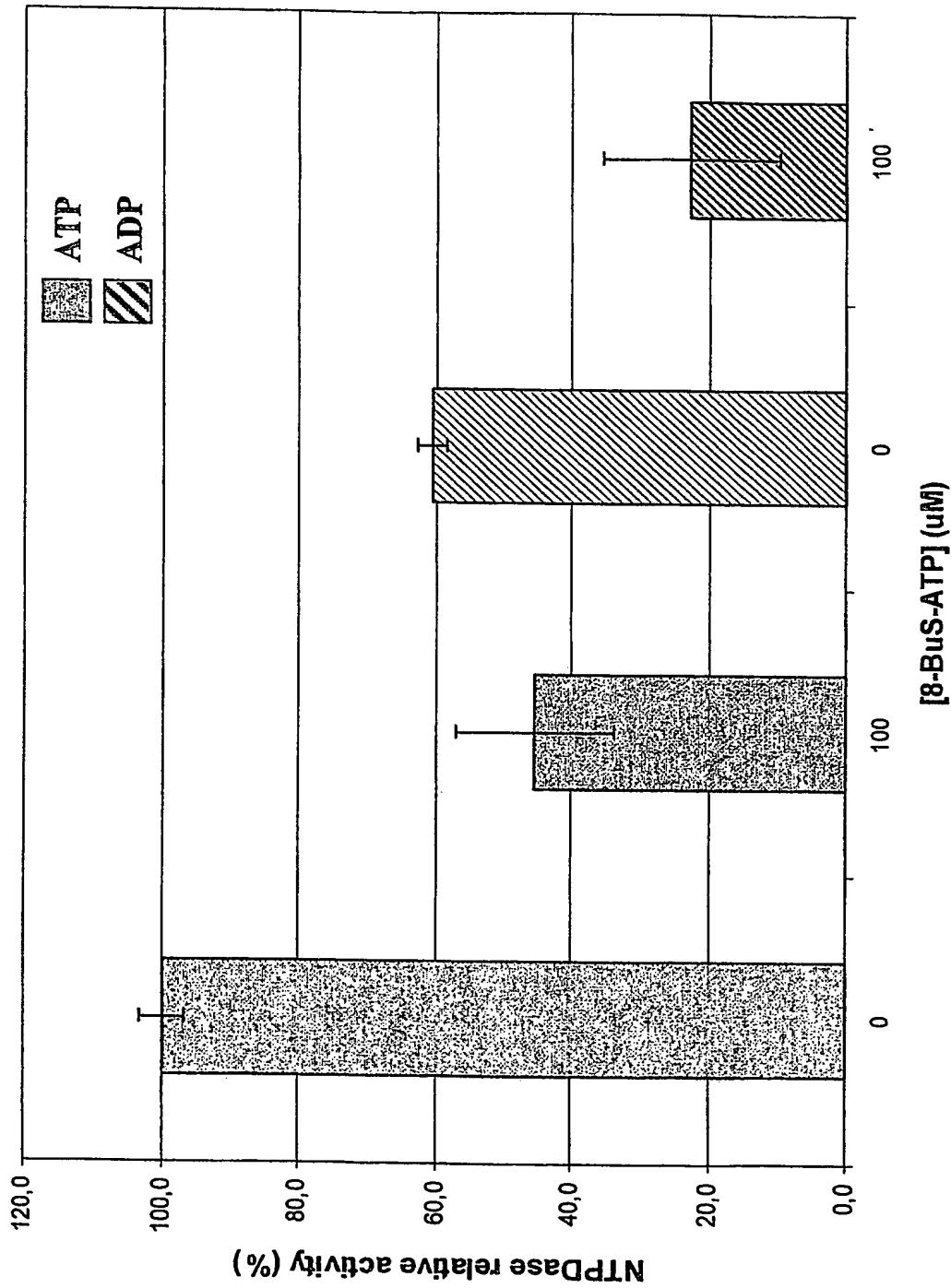
Effect of Erythrosin B on humoral response (antibodies production) 21 days after first injection



DM : Multiple doses of Erythrosin B were given (see methods)
 Groupe Ag : mice received only BSA as Ag T cell dependant without inhibitor
 Erythrosin B was given in a single dose as mentioned 0.25g/kg or 0.5g/kg
 Each group contain 10 mice

Fig. 9

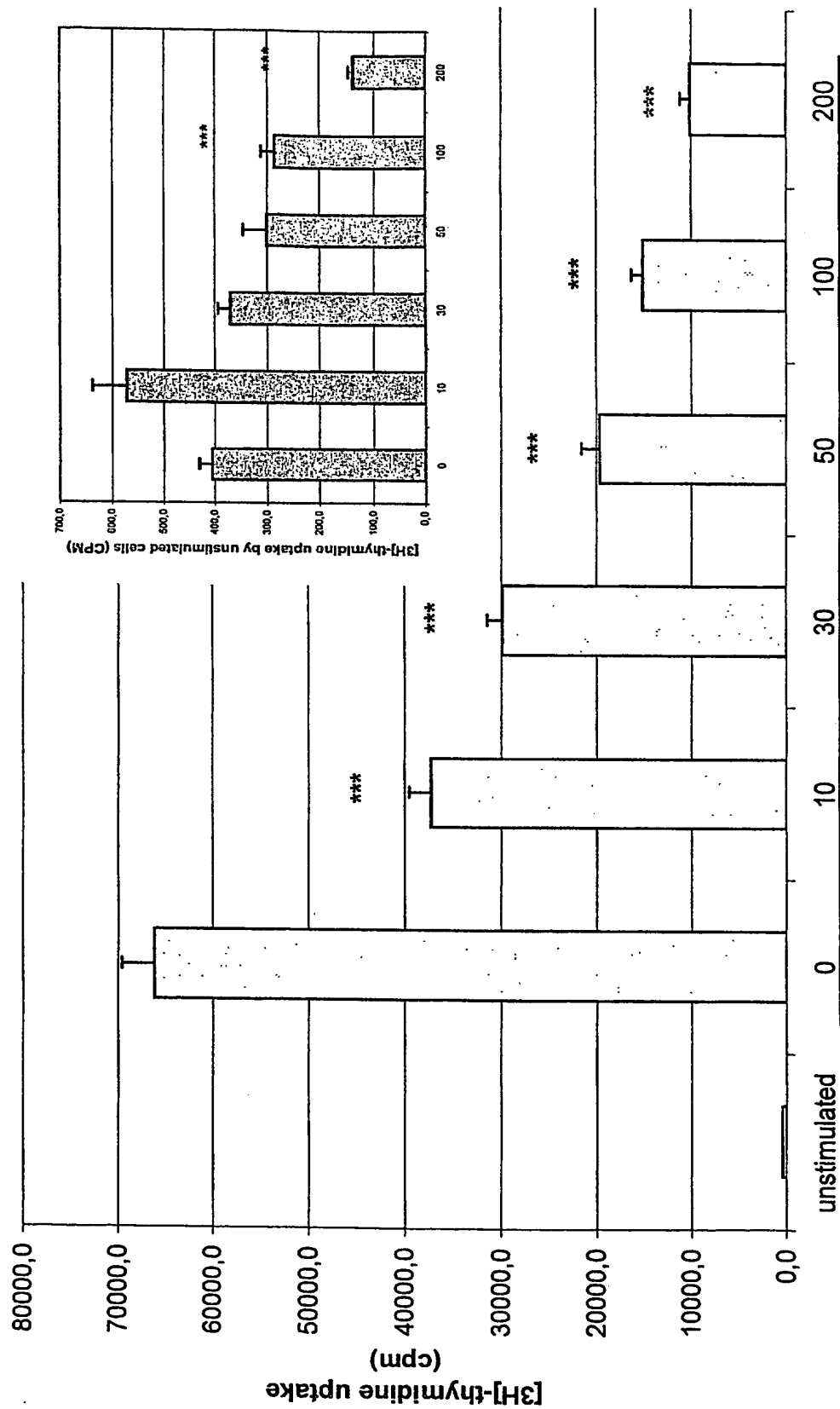
Inhibition of human peripheral blood lymphocyte NTPDase activity by 8-BuS ATP



One experiment was performed in duplicate in this case

Fig 10

Effect of 8-BuS-ATP on the human peripheral blood lymphocytes (PBL) proliferation

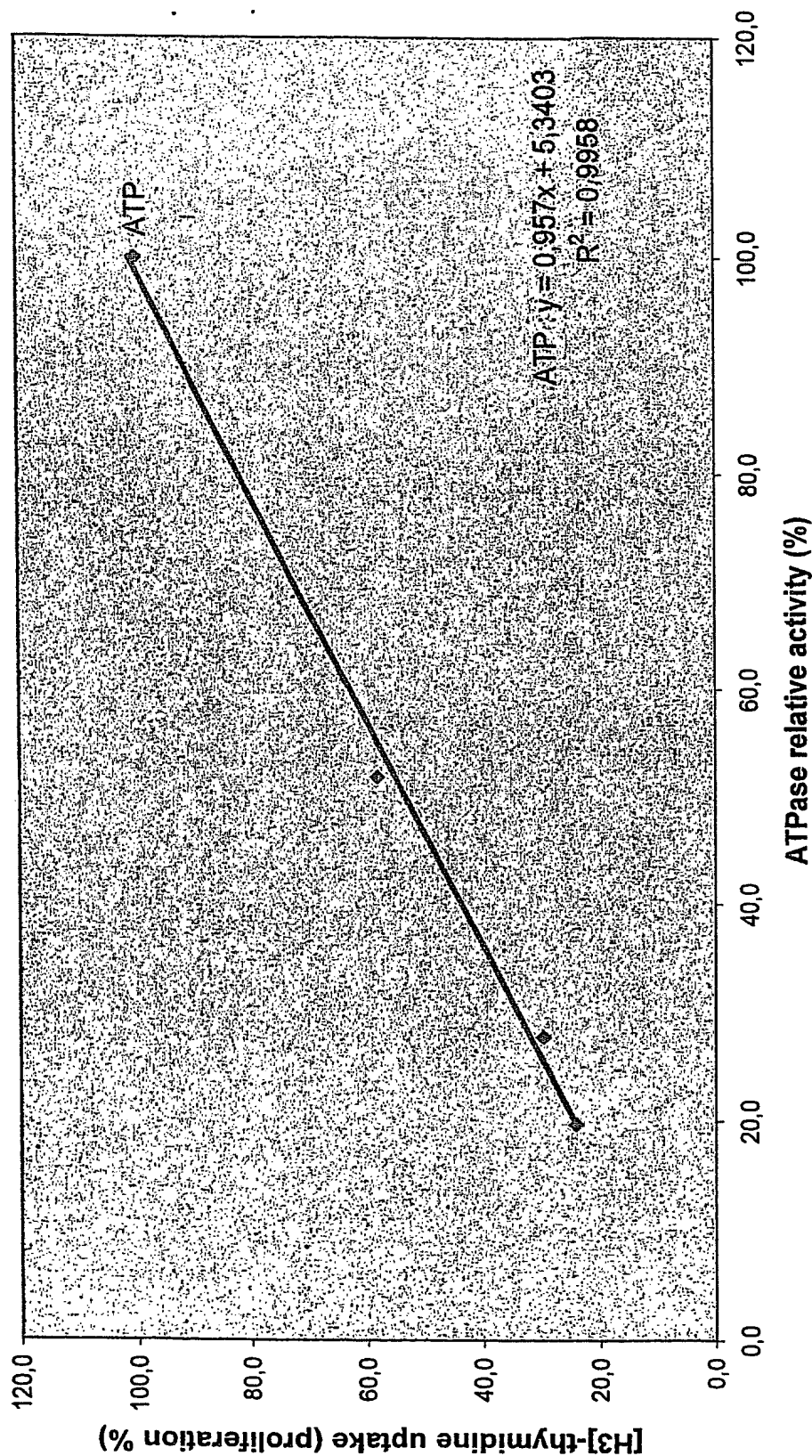


Stimulated cells by Con A +/- [8 BuS-ATP] (uM)

n = 3 ; n indicates the number of experiments performed each in sextuplicate

Fig. 11

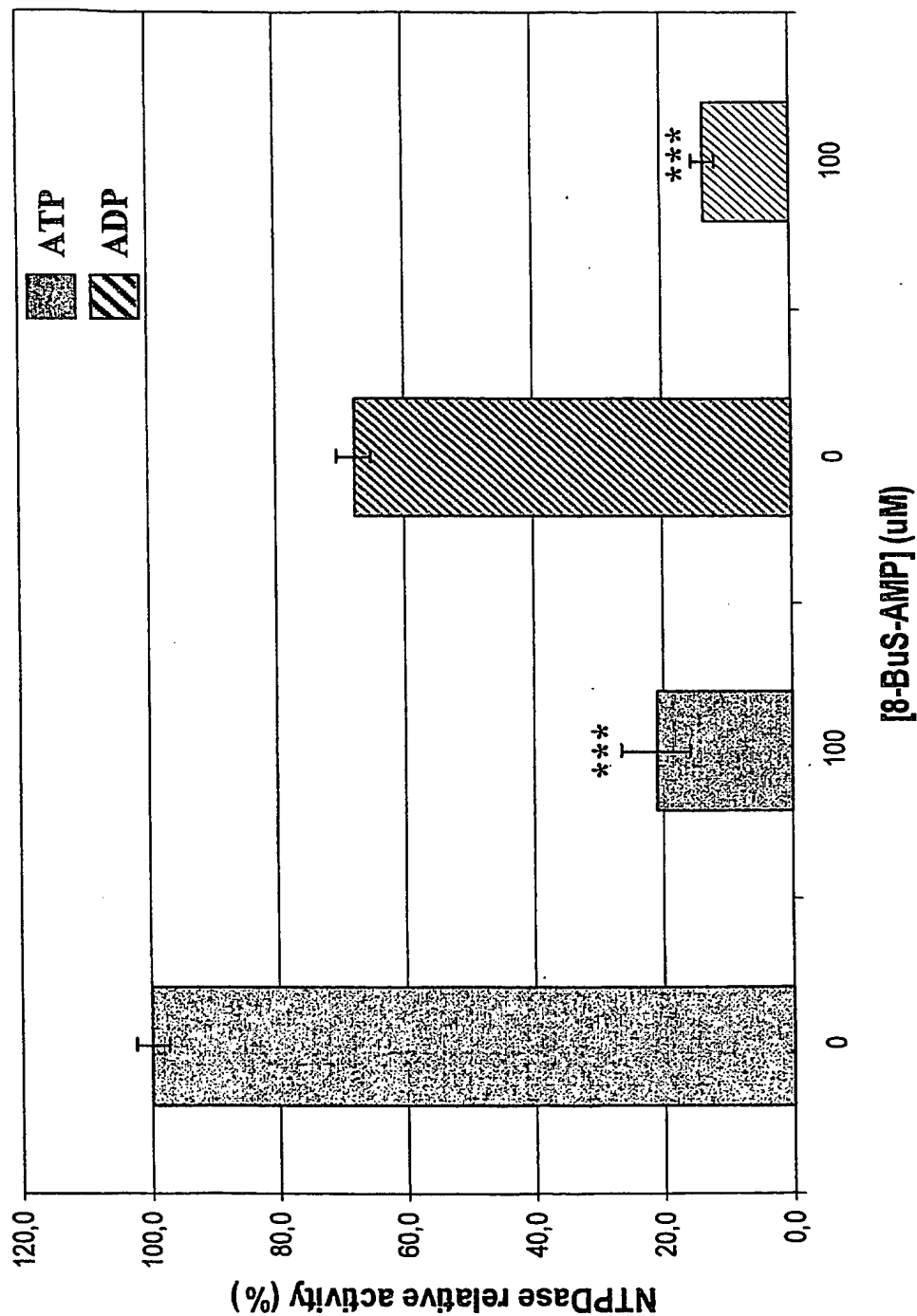
Correlation between NTPDase activity and proliferation assay of peripheral blood lymphocytes (PBL)



each set of point correspond to a single concentration of the inhibitor
(concentrations used are 0, 10, 50 and 100 uM of 8-BuS-ATP)

Fig. 12

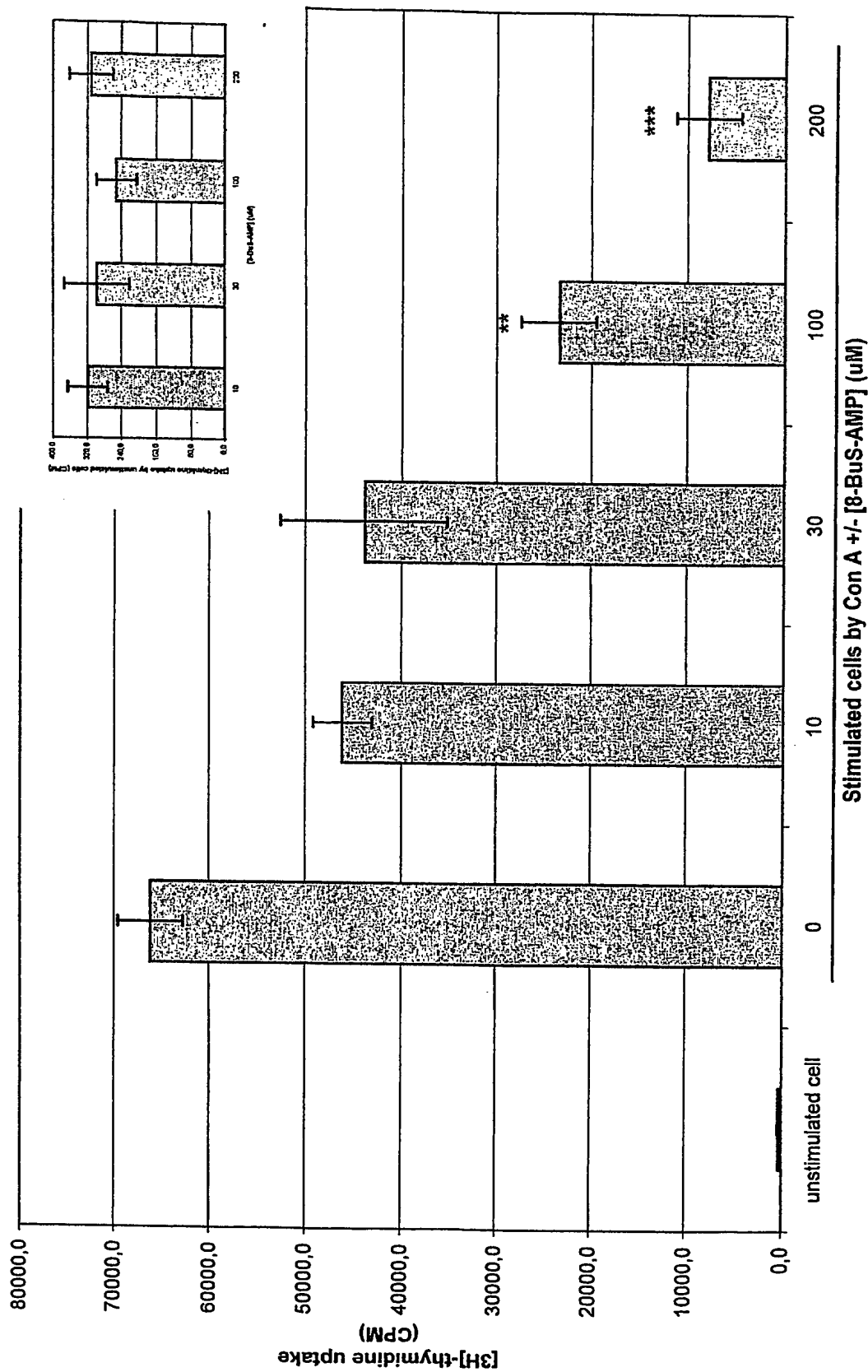
Inhibition of human peripheral blood lymphocyte NTPDase activity by 8-BuS AMP



n = 2 ; n indicate the number of experiments performed each in triplicate

Fig. 13

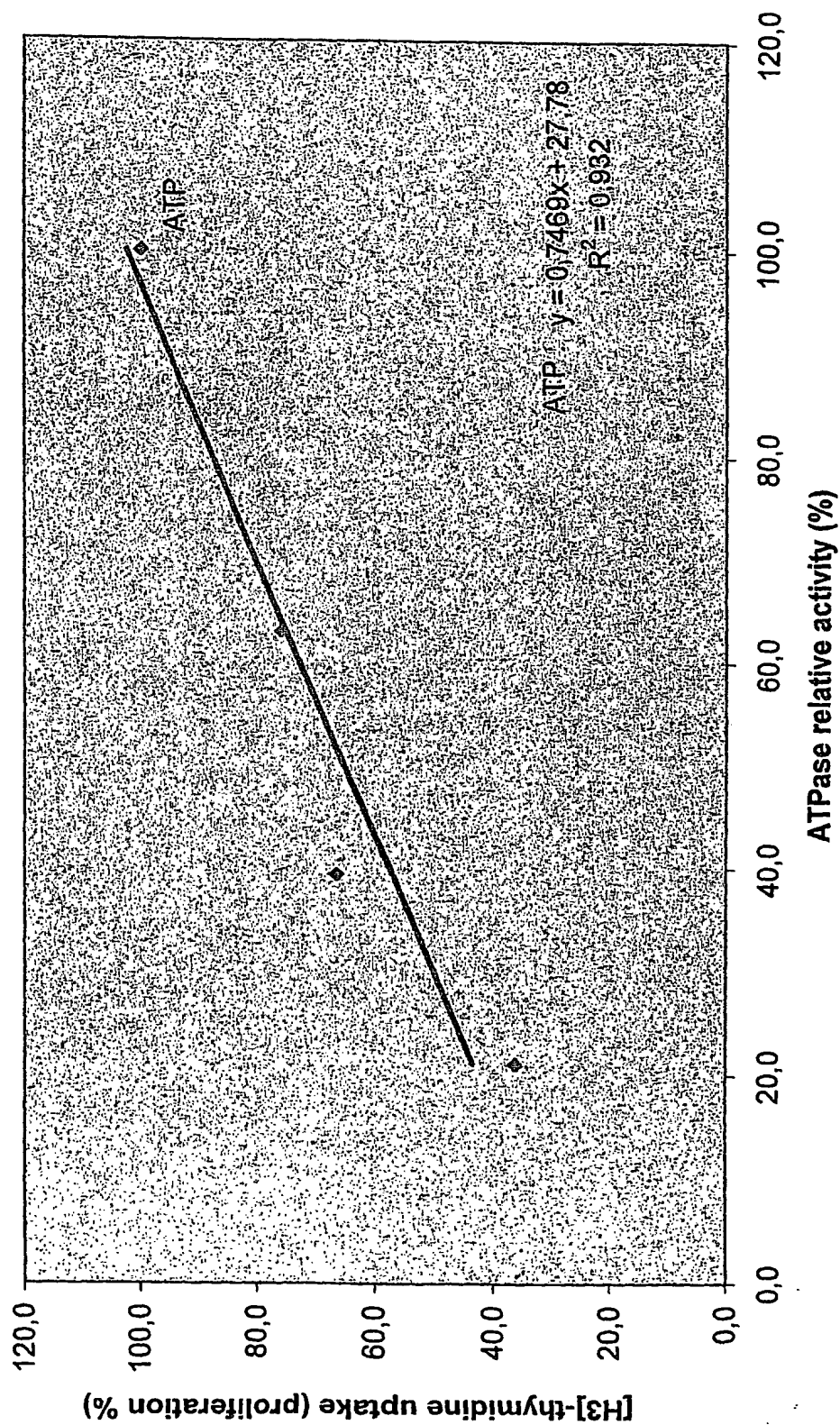
Effect of 8-BuS-AMP on the human peripheral blood lymphocytes (PBL) proliferation



n = 3 ; n indicates number of experiment each in sextuplicate

Fig. 14

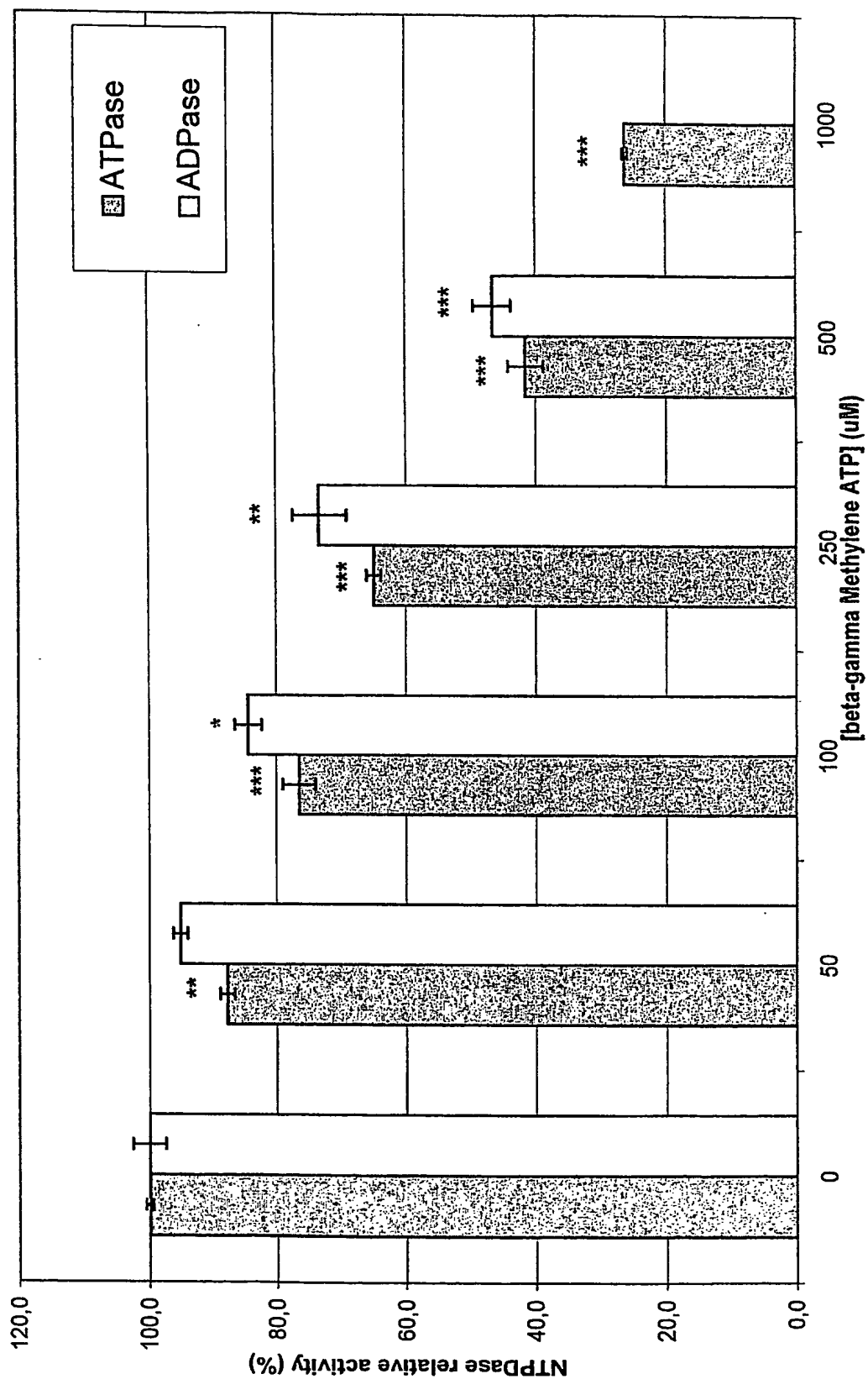
Correlation between NTPDase activity and proliferation assay of peripheral blood lymphocytes (PBL)



each set of point correspond to a single concentration of the inhibitor
(concentrations used are 0, 10, 30 and 100 uM of 8-BuS-AMP)

Fig. 15

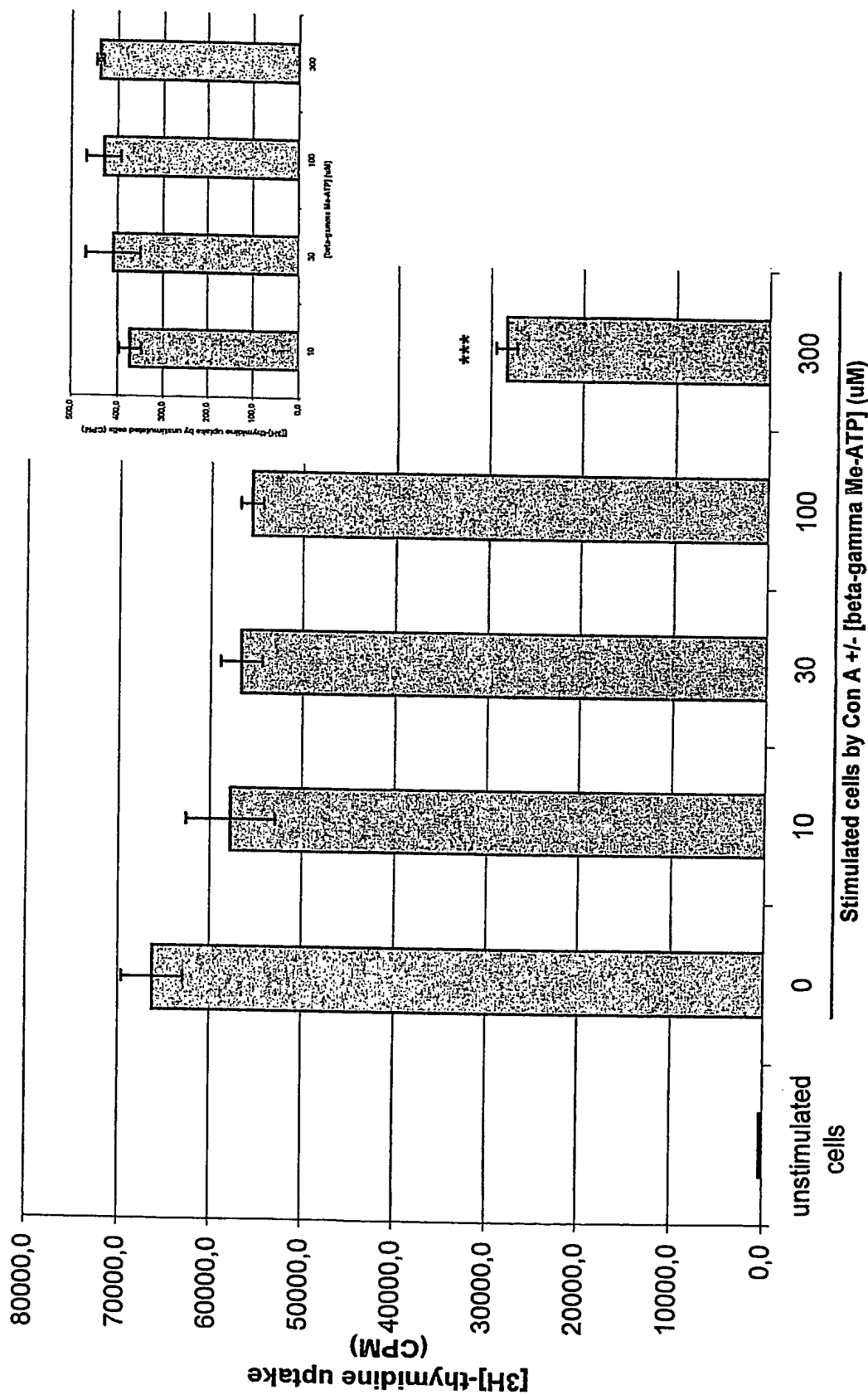
Effect of beta-gamma methylene ATP on NTPDase activity of human peripheral blood lymphocytes (PBL)



n = 2 ; n indicate the number of experiment performed each in triplicate

Fig. 16

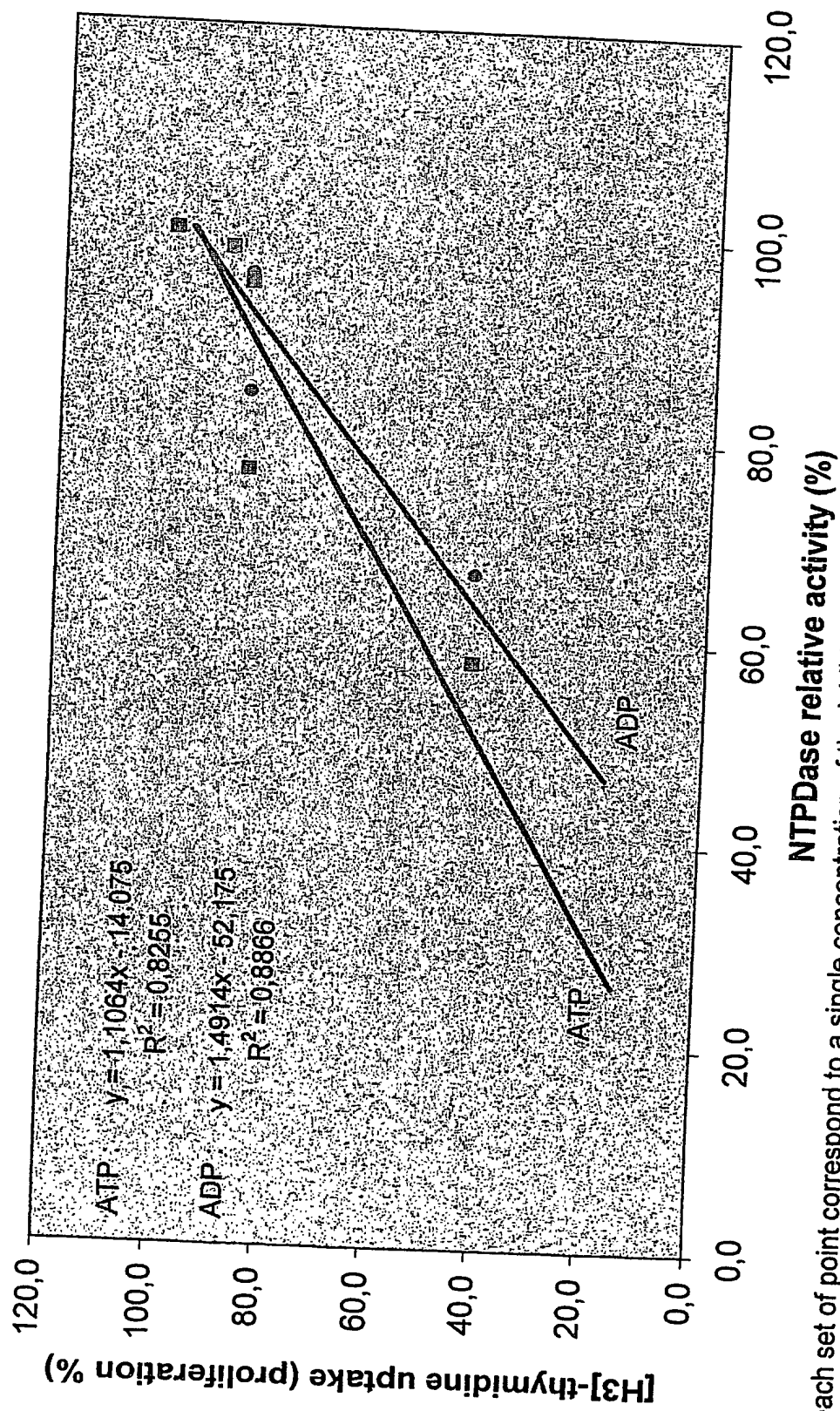
Effect of beta-gamma methylene ATP on the human peripheral blood lymphocytes (PBL) proliferation.



n = 3 ; n indicates number of experiment each in sextuplicate

Fig. 17

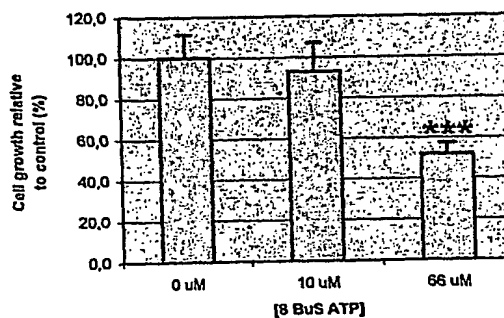
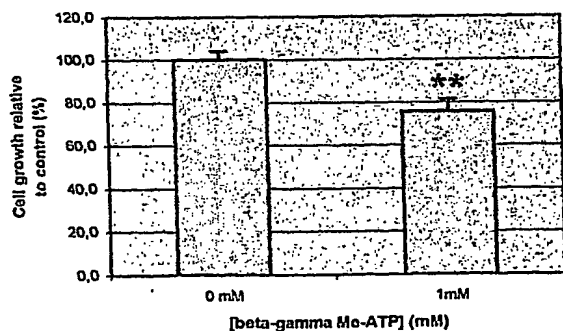
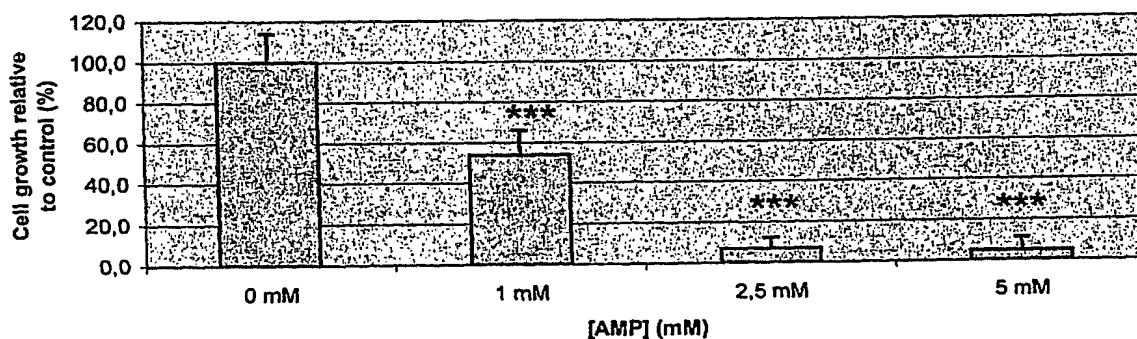
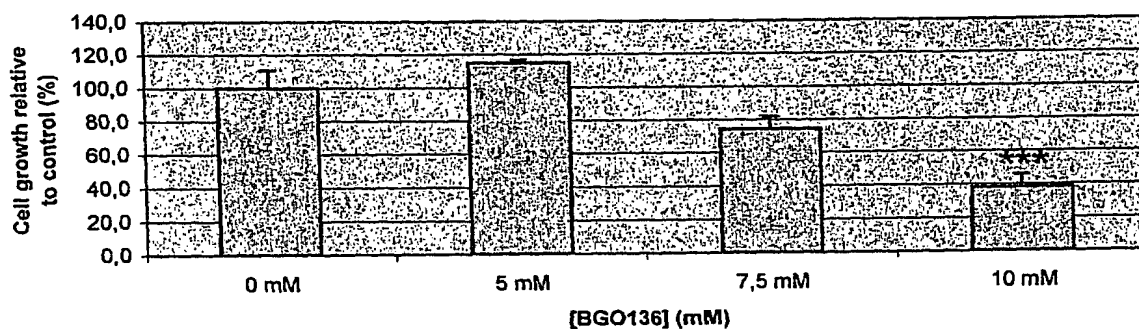
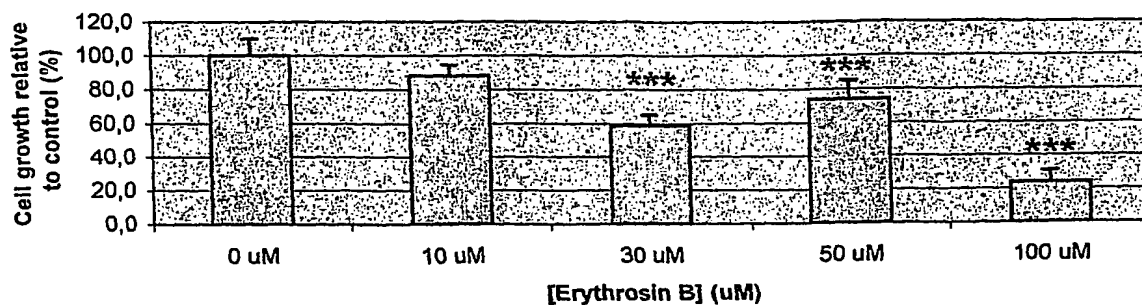
Correlation between NTPDase activity and proliferation assay of peripheral blood lymphocytes (PBL)



each set of point correspond to a single concentration of the inhibitor
(concentrations used are 0, 50, 100 and 250 uM of beta-gamma Me-ATP)

Fig 18

Effect of NTPDase inhibition on neoplastic T cell growth



For each inhibitor, two (2) experiments were performed each at least in quadruplicate

Fig. 19

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